Getting Plant Genes To Glow

New technique emerges from metal absorption studies

Studies of metal-absorbing plants by ARS scientists and a colleague have yielded an easier way to actually see where specific genes are expressed in plants.

The breakthrough—a new technique using the latest hybridization and microscopy technologies—was developed and tested recently at ARS's U.S. Plant, Soil, and Nutrition Laboratory at Cornell University, in Ithaca, New York. It may eventually help plant studies of all kinds.

"This enables us to quickly determine in which cells of a plant's root or shoot particular genes, regulating everything from micronutrient nutrition to heavy-metal transport, are expressed," says research leader Leon V. Kochian. "It provides immediate data that can be used in many ways."

Kochian explains that knowing where—in what tissues or organs—a gene and its product are expressed greatly helps researchers understand its role in plant function. "This is particularly important when studying plants' heavy-metal transport capabilities," he says. "If a gene is expressed only in cells at the root surface, that suggests it takes up metals from the soil." Plants that do this can be used to decontaminate toxic soils or extract metals via a process called "phytomining."

Kochian developed the new technique with Hendrick Küpper, a Humboldt Foundation postdoctoral fellow in his lab from the University of Konstanz in Germany, and ARS support scientist Laura Ort Seib. "It lets scientists work with large pieces of plant tissue that have been exposed to different environments, and eliminates many tedious, time-consuming steps associated with current processes for determining cell-specific localization of gene expression," he says. "Also, it's yielding valuable information about heavy-metal transporters' function in plants."

Expertise in an Emerging Science

To Kochian, data on heavy-metal transporter genes is of special interest. Over the past 19 years, he's become an international authority on the mechanisms used by certain plants to take up essential mineral nutrients and toxic heavy metals from soils.

Kochian has scientifically described strategies plants can use to tolerate toxic soils. He's an expert on plants' responses to environmental stress, their mineral nutrition, and their use for cleaning soils contaminated with heavy metals and radioisotopes. He's also explored how to keep toxic metals from the food chain.

With the new method, which was culled from existing techniques, a piece of a target gene's DNA is copied and then labeled with a fluorescent compound that's detectable with a laser scanning confocal microscope. The microscope portrays data in both visual and quantitative tabular forms. Kochian says a patent for the technique will not be sought.

"Generally, when a gene is expressed, an RNA sequence that's a mirror image of its DNA sequence is created," says Kochian. "This resulting nucleotide—known as messenger RNA (mRNA)—is the template for the protein that is subsequently synthesized."

Glowing Nucleotides as Markers

With the new procedure, a mirror image is made of the target gene's mRNA molecule. This synthetic nucleotide is then tagged with a fluorescent compound. The new, marked nucleotide binds tightly to the original mRNA molecule that's produced when the gene is expressed,

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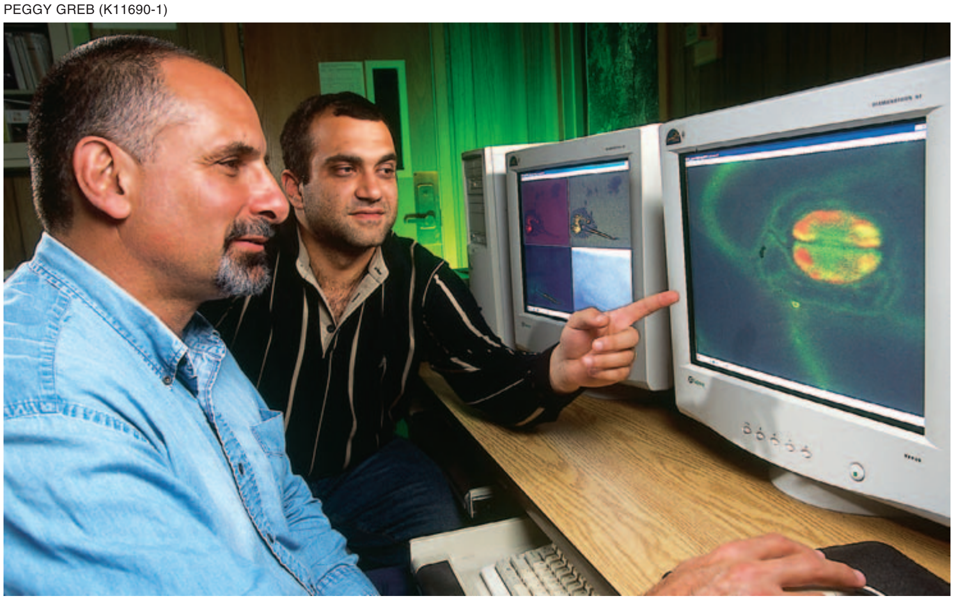

Research leader Leon Kochian (left) and Ph.D. student Ashot Papoyan look at a digital image of *ZNT1* gene expression in a pair of guard cells that make up stomata in the epidermis of a *Thlaspi caerulescens* leaf.

illuminating those cells where the target gene is functioning.

Currently, scientists trying to pinpoint gene expression in plants must fix and dehydrate plant tissue, embed it in plastic or resin, and then slice it into thin sections. These sections are then attached to a slide and marked with a probe that binds a fluorescent agent or a labeled antibody to the mRNA for a specific gene.

This sectioned tissue is usually viewed under an electron microscope to determine which cells within it are labeled. The labeling indicates where the gene is expressed. "This is quite labor intensive and requires specialized microscopy skills," says Seib. "Also, it's often beset with problems, such as degradation of target mRNA and labeling material by RNA introduced during hybridization.

"The new approach is much easier to use, so it dramatically increases the information we can process," she says. "A major time saver is that a confocal laser scanning microscope makes much of the sectioning unnecessary."

One of two major forms of laser scanning microscopy, confocal laser scanning microscopy uses a beam of laser light focused into a small point of a specimen and can be moved with a computer-controlled scanning mirror. The detected input is displayed in a computer-generated image.

Findings With Alpine Pennycress

The new method has already led the Ithaca researchers to significant findings regarding alpine pennycress, *Thlaspi caerulescens*. This wild plant, found mainly in Rocky Mountain and western states, has been cited as a hyperaccumulator of nickel, cadmium, and zinc.

The group initially used the method to study the expression of *ZNT1*, an important zinc transporter gene, in alpine pennycress leaves. They found that in young leaves, *ZNT1* mRNA was abundant in the bundle sheath of the veins, adjacent mesophyll cells, and guard cells of the epidermis.

"Because metal hyperaccumulation in *T. caerulescens* occurs specifically in large epidermal cells, this expression data indicates that *ZNT1* does not participate in metal hyperaccumulation within the leaf," says Kochian. "Instead, it probably plays a role in the leaf's normal zinc nutrition. This runs contrary to previously published reports that suggest that *ZNT1* plays a key role in metal hyperaccumulation in *Thlaspi*."

Kochian sees many potential uses for the new technique in studies of gene function in any plant.

"It'll make it possible for biologists with no significant training in microscopy to determine the cellular location of expression of their favorite gene. That will provide useful information about the role of this gene in plant function and performance," he says. "Also, because the technique is not labor intensive, researchers will be able to study the localization of gene expression of a significant number of genes, such as different members of a gene family, which wasn't easy to do with more conventional methods."—By **Luis Pons,** ARS.

This research is part of Plant Biological and Molecular Processes, an ARS National Program (#302) described on the World Wide Web at www.nps.ars.usda.gov.

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Ph.D. student Melinda Klein harvests leaves from *Thlaspi caerulescens* for analysis by a technique called "quantitative in situ hybridization." The method can spot specific cells in which the gene for heavy metal transport is expressed.

SCOTT BAUER (K8784-10)

Hyperaccumulators like *Thlaspi* possess genes that regulate the amount of metals taken up from soil by roots and deposited at other locations within the plant.